

A potential application for the intracerebral injection of drugs entrapped within liposomes in the treatment of human cerebral gliomas

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SUMMARY After intravenous injection of therapeutic doses of bleomycin only small amounts could be measured in glioma tissue obtained at operation in patients with malignant gliomas and the drug was rapidly cleared from the blood ($T_{1/2} = 2$ hrs). Negatively charged liposomes injected through Ommaya reservoirs into the glioma tumour bed were tolerated without observable side effect. The appearance of bleomycin in the blood and urinary clearance after intracerebral injection of bleomycin entrapped within negatively charged liposomes through an Ommaya reservoir in patients with malignant gliomas was decreased as compared with levels when free bleomycin was injected. These preliminary observations suggest a role of drugs entrapped within liposomes as a depot preparation in the treatment of human cerebral gliomas.

Surgery, radiotherapy and more recently chemotherapy and immunotherapy have improved the outcome in the treatment of human cerebral gliomas to some extent but the sad fact remains that the condition is still uniformly fatal with poorly differentiated gliomas having a reported median survival time of 5–13 months.¹ Lipid soluble drugs which readily cross the normal blood-brain barrier have been found to be generally more effective than those which are less lipid soluble and more often cell-cycle specific. This is particularly unfortunate since in no other type of cancer is there such a clear kinetic difference between the tumour and the normal tissue with respect to its cell division rate.² If cell cycle specific drugs could be delivered in an effective and sustained concentration a good differential response might be anticipated. However to achieve a significant concentration of drug for a therapeutically adequate period of time in the central core of a 6 cm diameter glioblastoma multiforme at a depth of

1–3 cm from the perfused rim, it has been calculated that a therapeutic plasma level of drug would have to be maintained for 10–24 h.³ Since many of the cell cycle specific drugs have relatively short half lives after intravenous injection to maintain sustained therapeutic levels of drug in the blood would result in unacceptable side effects.

Local injection of antimitotic drugs into cerebral tumours has been used but the vascularity of the glioma tissue bed is such that high enough concentrations of drug cannot be sustained for an adequate period of time.⁴ These considerations suggest that an inert depot preparation of antimitotic drug injected locally into the tumour mass with sustained release of drug might be more effective than a similar dose of drug given systemically and should enable a higher dose of drug to be given without the appearance of systemic toxicity.

We have recently determined the optimal conditions for producing the maximum incorporation within negatively charged liposomes of two relatively cell cycle specific drugs (bleomycin and vincristine) used in the treatment of human cerebral gliomas.⁵ A delayed release of these drugs from the liposomes was demonstrated and dose response curves were similar for the two drugs whether given as the free or liposome entrapped drug to a human

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glioma cell line (U251-MG). Subsequent studies on the intracerebral injection of bleomycin free and entrapped within liposomes in the rat demonstrated no detectable cerebral toxicity after injection of liposomes, and a delayed clearance of the drug from the injection site when given entrapped within liposomes rather than as the free drug.⁶ A similar delayed cerebral clearance was demonstrated with vincristine although when given either as the free or liposome entrapped drug cerebral toxicity was present.⁷ These animal experiments have suggested a potential application for drugs entrapped within liposomes acting as a depot preparation in the treatment of human cerebral gliomas. We have now extended these studies to human subjects with malignant gliomas and report our preliminary observations.

Patients and methods

Materials

Cholesterol, dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidic acid of highest obtainable purity were purchased from Sigma Chemical Company. Bleomycin was kindly donated by Lundbeck Ltd. Sepharose 6B was obtained from Pharmacia Ltd. Bleomycin antisera and donkey antisherp serum were obtained from Guildhay, University of Surrey. ¹²⁵I was obtained from Amersham International. All other reagents were obtained through BDH Ltd.

Methods

Iodination and radioimmunoassay (RIA) of bleomycin. Iodination was performed using the method of Salacinski *et al.*⁸ The RIA was performed as previously described.⁶ The technique had an interassay coefficient of variation of 3.5% and an intra-assay coefficient of variation of 7.5% at a level of 50 ng/ml, and a detection limit of 40 pg, per tube.

Preparation of liposomes Liposomes were prepared by the reverse phase method described by Szoka and Papahadjopoulos.⁹ Standard sterile tech-

niques were adopted throughout. Samples from each preparation were sent for microbiological examination and cultured for 48 h prior to human intracerebral injection, during which time the liposomes were stored at 4°C to reduce the rate of release of free drug.⁵ At the time of human administration the amount of free drug released from the liposomes was determined in an aliquot by separation on sepharase 6B.

Assay of bleomycin in human cerebral glioma tissue In four patients with malignant gliomas (Grade IV astrocytomas) and one with a cerebral secondary deposit, cerebral tumour tissue at different depths was taken at routine craniotomy for the surgical treatment of the tumour 15 and 30 mins after intravenous injection of 10 mg of bleomycin. The tumour was immediately stored at -20°C until assayed for bleomycin. The specimens were homogenised in an 0.05M pH 7.4 phosphate buffer using a glass hand held homogeniser and assayed by RIA for bleomycin. Concentrations were expressed in terms of concentration of bleomycin/mg of wet tumour tissue.

Patients These studies have been approved by St George's Hospital District Ethical Committee. Three patients with malignant cerebral gliomas were studied. Blood levels and the urinary clearance of bleomycin after intravenous injection of 5, 10 and 15 mg of bleomycin were determined in subject 1 over 24 h. In subject 2 the blood levels and urinary clearance of bleomycin after intravenous injection of free and liposome entrapped drug was studied over 6 h. Negatively charged liposomes were injected through an Ommaya reservoir inserted at previous craniotomy into the tumour bed in subject 1 and her behaviour carefully monitored over a 2 week period. Increasing amounts of free bleomycin were injected through an Ommaya reservoir and blood and urine levels measured over 18 h in subject 3. Liposome entrapped bleomycin was injected through an Ommaya reservoir into the tumour bed in subjects 1 and 3, and blood and urine levels of drug determined.

Table 1 Tumour levels of bleomycin following intravenous injection of 10 mg bleomycin

	ng bleomycin/mg wet wt brain tissue					
	Surrounding margin of macroscopically Viable tumour normal tissue				Necrotic centre of the tumour	
	15 min	30 min	15 min	30 min	15 min	30 min
Patient A	<0.03	<0.03	—	—	0.13	0.13
Patient B	0.09	0.11	0.23	0.26	0.19	0.12
Patient C	<0.10	0.12	0.29	0.21	0.13	0.19
Patient D	<0.05	0.43	<0.06	0.48	<0.2	0.42
Patient E	0.66	0.41	—	—	0.24	0.21

Table 2 Peak serum bleomycin levels achieved following injection of bleomycin, free and entrapped within liposomes

Dose of bleomycin injected (mg)	Serum bleomycin (ng/ml)			
	Intravenous injection*		Intracerebral injection	
	Free	Entrapped	Free	Entrapped
0.1 mg			2.6 ¹	<1.0 ¹
0.45 mg			4.6 ¹	1.1 ³
2 mg		108 ²	41.5 ³	3.6 ³ /4.0 ³
3.3 mg	617 ²			
5 mg	500 ¹			
6 mg			66.0 ³	
10 mg	1200 ¹			
15 mg	2034 ¹			

*Levels achieved 5 minutes after intravenous injection.

1 = Patient 1, 2 = Patient 2, 3 = Patient 3.

Table 3 24 hour urinary excretion of bleomycin following injection of bleomycin, free and entrapped within liposomes

Dose of bleomycin injected (mg)	24 hour urinary bleomycin excretion (% of total)			
	Intravenous injection		Intracerebral injection	
	Free	Entrapped	Free	Entrapped
0.1 mg			12 ³	ND ¹
0.45 mg			26.5 ³	11.1 ³
2 mg		39.5 ²	79.5 ³	11.2 ³ /18.6 ³
3.3 mg	91 ²			
5 mg	80 ¹			
6 mg			60.0 ³	
10 mg	86 ¹			
15 mg	75 ¹			

ND = Not detected

1 = Patient 1, 2 = Patient 2, 3 = Patient 3.

Results

Tumour levels of bleomycin after intravenous injection

The tumour levels of bleomycin at different depths in the tumour after intravenous injection of 10 mg of bleomycin 15 and 30 mins before sampling are shown in table 1. In the subject with a cerebral secondary the lowest levels of bleomycin were found probably owing to the known presence of a bleomycin hydrolase in these non-ectodermally derived neoplastic cells.¹⁰ With one exception there was no difference in the amount of bleomycin which had permeated the tumour tissue at 30 as compared with 15 mins. The highest concentration of drug was found in the area of viable tumour as compared with the surrounding margin of macroscopically normal tissue or the necrotic centre of the tumour. The concentration of drug achieved in the tumour was between $\frac{1}{4}$ and $\frac{1}{2}$ that obtained in blood at 30 mins.

Blood and urine levels of bleomycin achieved after intravenous injection in subject 1 of the free drug and in subject 2 of both the free and liposome entrapped drug.

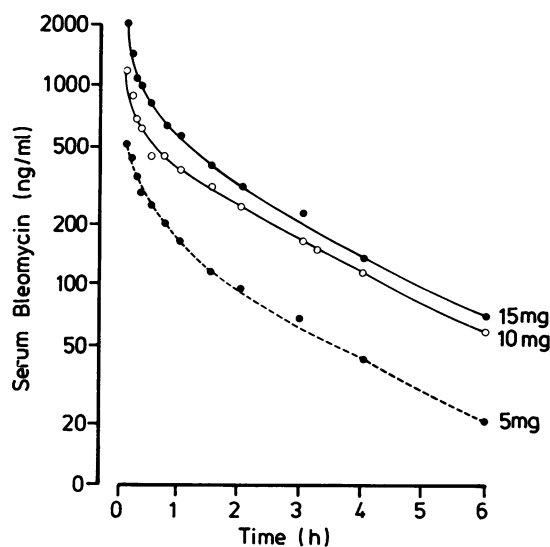


Fig.1 Bleomycin clearance from blood following intravenous injection in a human subject with a malignant cerebral glioma (patient 1).

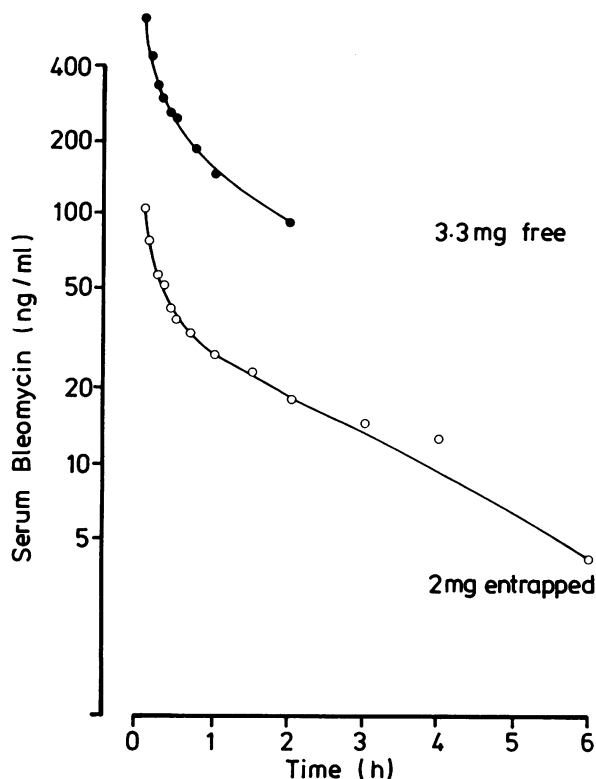


Fig. 2 Serum bleomycin levels following intravenous injection of bleomycin free and entrapped within liposomes in a subject with a malignant glioma (patient 2).

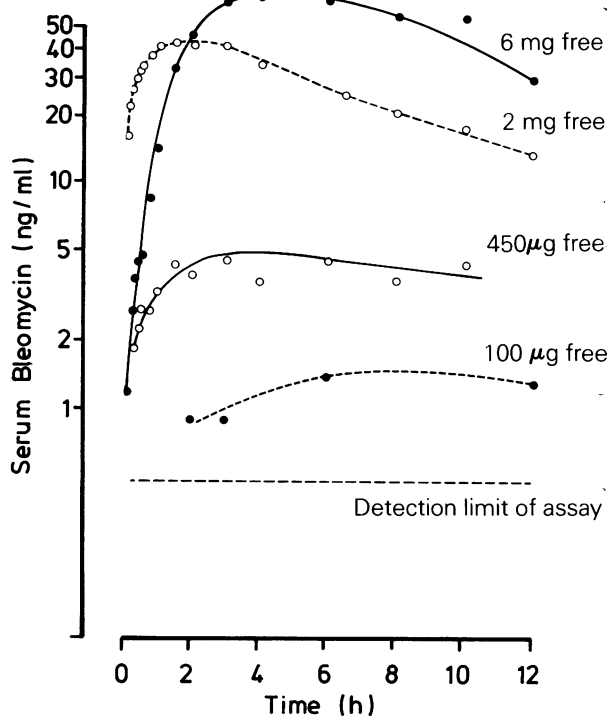


Fig. 3 Serum bleomycin levels following intracerebral injection of free bleomycin in a subject with malignant gliomas (patient 3).

The blood and urine levels of bleomycin achieved after intravenous injection of 5, 10 and 15 mg of bleomycin in subject 1 are shown in Figure 1 and tables 2 and 3. The drug $T_{1/2}$ in blood was 112 min (range 110–116 min) and urinary clearance 80% (range 75–86%) at 24 h.

In subject 2 the peak level in the blood after intravenous injection of bleomycin entrapped within liposomes was considerably less than when the free drug was given (fig 2) and the urinary clearance approximately half that obtained with the free drug (40% as compared to 91% at 24 h).

Blood and urine levels of bleomycin after intracerebral injection of bleomycin free and entrapped within liposomes through an Ommaya reservoir in subjects 1 and 3.

No adverse effect was recorded clinically in subjects 1 and 3 over a total period of 4 weeks observation which could be attributed to the effect of injecting

negatively charged liposomes into the tumour. Their conscious level, the clinical evidence of raised intracranial pressure and focal neurological deficits remained unaltered and they recorded no headache or drowsiness and no temperature or seizures were witnessed.

The blood levels and urinary excretion of bleomycin after the injection of the free drug through the Ommaya reservoir into the tumour in subject 3 is shown in figs. 3 and 5. The urinary clearance of bleomycin increased as the amount of drug injected increased and at 6 mg approached the levels recorded after intravenous injection of free drug. Blood and urine levels of bleomycin after injection of the liposome entrapped drug through an Ommaya reservoir into the tumour in subjects 1 and 3 are shown in figs 4 and 5. With 100 µg of bleomycin entrapped within liposomes no blood level or urinary clearance could be demonstrated although this was possible after the intratumour injection of

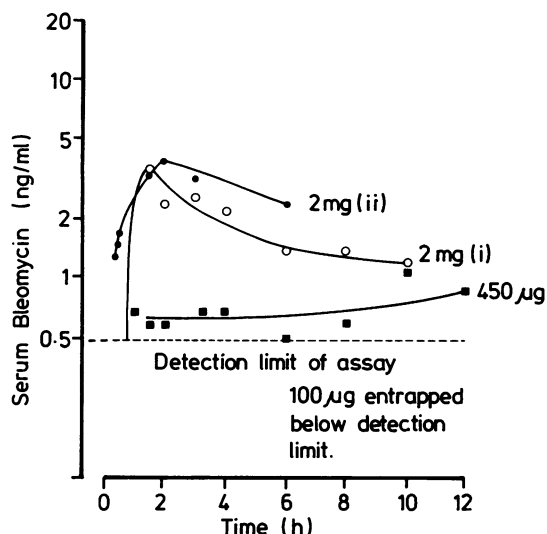


Fig. 4 Serum bleomycin levels following intracerebral injection of bleomycin entrapped within liposomes in 2 subjects with malignant gliomas (patients 1 and 3).

the free drug. The blood levels were reduced several fold and the urinary clearance to between a $\frac{1}{4}$ and a $\frac{1}{2}$ when bleomycin was given entrapped within liposomes as compared to the free drug. The $T_{1/2}$ in blood for bleomycin when injected into the tumour bed was approximately 3 times that when given intravenously (6 h as compared to 2 h). The $T_{1/2}$ in blood for bleomycin when injected into the tumour bed and entrapped within liposomes was difficult to determine owing to the low levels encountered in blood and the fact that some free drug that had leaked out of the liposomes in storage would account for some of the early appearance of bleomycin in the blood.

Discussion

The dynamics of brain tumour growth are complex and consist of many mutually interacting phenomena which should be, although rarely are, taken into account when considering the therapeutic goal of reducing and if possible eradicating the neoplastic cells.¹¹ Since in addition so many factors have to be considered in the delivery of antimitotic drugs to cerebral neoplasms¹² it is perhaps not surprising that the results of systemic chemotherapy in the treatment of gliomas has been disappointing. The biological variation in the behaviour of cerebral gliomas of different histological type also suggests that each group must be considered separately with respect to planning chemotherapy and assessing

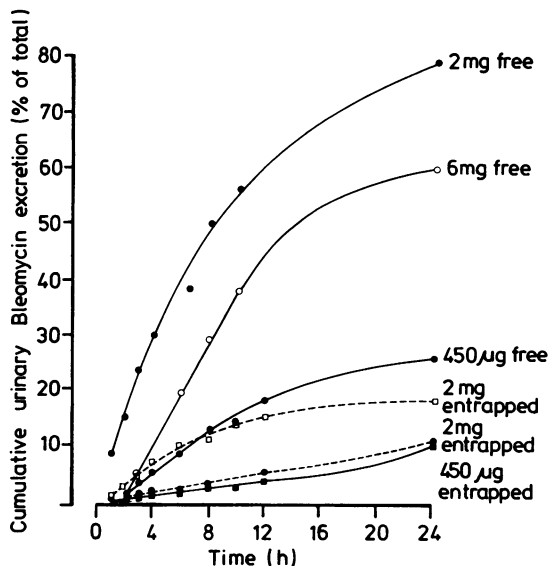


Fig. 5 Urinary excretion of bleomycin following intracerebral injection of bleomycin free and entrapped within liposomes in a subject with a malignant glioma (patient 3).

response to treatment.¹³

Liposomes have recently been extensively studied as possible vehicles for delivering drugs to the required site of action in animal and human experiments.¹⁴ It has been demonstrated that liposomes of defined composition can be safely injected intracerebrally in the mouse¹⁵ and rat^{6,7} although certain types of liposome are toxic.¹⁵ The effectiveness of drugs entrapped within liposomes in cancer chemotherapy may be enhanced by recent developments that have enabled them to be targeted to the desired site by coupling antibody to the liposome surface.¹⁶

Bleomycin has been extensively studied as a chemotherapeutic agent with some success in the treatment of human ectodermally derived tumours.¹⁷ It is an antibiotic derived from *Streptomyces verticillus* shown to be particularly effective against certain types of cancer (skin, head and neck) and especially squamous cell carcinoma. Bleomycin when given intravenously to mice has a significant anti-tumour effect on gliomas with some preferential uptake into the tumour, but at high enough concentrations to produce complete cessation of tumour growth the animals lose weight and experience significant side effects.¹⁸ Similarly, high and sustained dosage of bleomycin in human cancer chemotherapy results in cutaneous and pulmonary complications. Since the major mode of action of bleomycin is to produce scission of DNA strands

and thus hinder cell division¹⁹ it might be particularly useful in the treatment of human cerebral gliomas by acting on dividing gliomatous tissue with less effect on non-dividing neural tissue. These considerations suggest that an inert depot preparation of bleomycin injected locally into a tumour mass with sustained release of drug might be more effective than a similar dose given systemically and would enable a higher dose to be given with less likelihood of systemic toxicity.

We have confirmed the observations of Hayakawa *et al*²⁰ that bleomycin is taken up by human glioma tissue *in vivo* after intravenous injection of therapeutic doses but at relatively low concentration. Dose response curves *in vitro* of a human glioma cell line (U251-MG) to bleomycin demonstrated that it was an effective chemotherapeutic agent but at relatively high concentration.⁵ The rapid clearance of bleomycin from the blood after intravenous injection indicated that high sustained levels of bleomycin at the tumour site could not be maintained without the likelihood of systemic side effects. Our preliminary observations have indicated that negatively charged liposomes of defined composition can be safely administered to human subjects with malignant gliomas through Ommaya reservoirs direct to the tumour bed without significant side effect. Although the desired high concentrations of bleomycin at the glioma tissue bed can be achieved by direct injection of the free drug through an Ommaya reservoir into the tumour, these studies suggest frequent injections would be required to maintain adequate therapeutic doses. However when bleomycin is injected into the glioma tumour bed entrapped in liposomes the blood levels achieved are approximately 10% of those obtained after injection of the free drug and the urinary clearance reduced. These results together with our *in vitro* studies on the rate of release of bleomycin from liposomes⁵ suggest that bleomycin can be given at high dosage entrapped within liposomes to act as a slow release depot preparation enabling high infrequent repeated dosages of drug to be given over a protracted treatment programme. We are currently refining and optimising an appropriate treatment schedule.

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